Hormones and Dietary Fat as Promoters in Mammary Carcinogenesis

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Hormones, particularly ovarian steroids and pituitary prolactin, promote mammary carcinogenesis in rats treated with a carcinogen. Hormones also play a critical role during the initiation process as demonstrated by mammary carcinogenesis in ovariectomized rats. A diet high in fat content, especially polyunsaturated fat, promotes mammary tumorigenesis when it is fed to carcinogen-treated rats for a prolonged period of time. Although a high fat diet is not essential for neoplastic transformation of the mammary cells, its effect on initiation is demonstrated when it is fed to rats for a long duration. Thus, both hormones and high dietary fat play a dual function in mammary carcinogenesis. There are indications that dietary fat may modulate endocrine activities, but a relationship between dietary fat and endocrine function remains to be conclusively demonstrated.

Introduction

This paper presents an overview of the role of hormones and dietary fat as tumor promoters in mammary carcinogenesis in the rat. Several informative reviews on this subject have been published (1,2). To state that hormones, particularly the steroidal estrogens, and dietary fat are mere tumor promoters is an oversimplification at best. The critical role of ovarian hormones in the initiation of mammary carcinogenesis by a chemical carcinogen has been reported earlier (3). The working hypothesis that hormones, particularly the estrogens, play a dual function in mammary carcinogenesis is as valid today as it was when first suggested by Dao (4) about a decade and a half ago.

Similarly, the concept that a high fat diet enhances mammary carcinogenesis merely by its promotional effect can be challenged. Recent experiments from our laboratory strongly suggest that the enhancing effect of dietary fat on mammary tumorigenesis is proportional to the duration of time for which the rats are on the high fat diet, irrespective of whether the high fat diet is given before or after the carcinogen treatment (5).

These observations on the effects of hormones or a high fat diet clearly suggest that hormones or a high fat diet are important cofactors modulating not only the initiation process but also the expression of

transformed cells to form tumors and the rate of subsequent tumor growth. It is suggested that investigations must be directed toward examining the possible interactions between the carcinogen and hormones and between the carcinogen and dietary fat in both the initiation and promotion of mammary carcinogenesis. It is this perception that leads us to look first at a possible relationship between dietary fat and endocrine functions in the induction of mammary tumors by a chemical carcinogen.

Tumor Promotion in the Mammary Gland by Hormones

The best examples of tumor promotion by hormones are experiments designed to study the effects of pregnancy and the effects of ovariectomy on mammary carcinogenesis in the rat.

Effects of Pregnancy

The study of the effects of pregnancy in the induction of mammary tumors by a chemical carcinogen demonstrated, perhaps for the first time, the possible presence of a two-stage mechanism in mammary carcinogenesis. Dao and Sunderland (6) reported that in rats previously treated with a chemical carcinogen, such as 3-methylcholanthrene (3-MCA), pregnancy greatly accelerated the appearance and incidence of mammary tumors. Figure 1 is a schematic diagram based on data published earlier to illustrate the effect of pregnancy on the promotion of mammary tumorigenesis by 3- methylcho-

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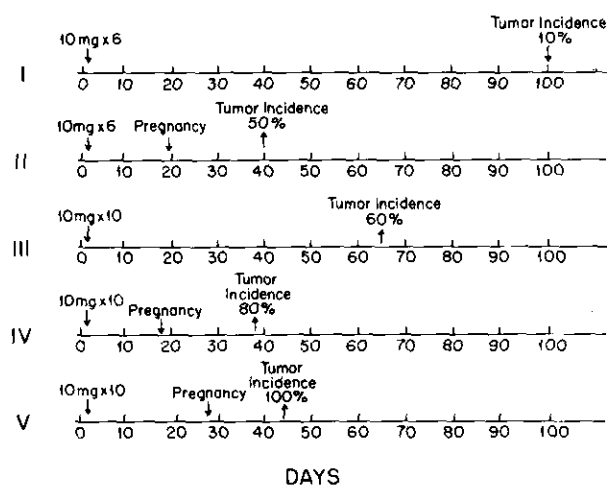
FIGURE 1. Promoting effect of pregnancy in mammary carcinogenesis by 3-methylcholanthrene in Sprague-Dawley rats.

FIGURE 2. Relationship between time elapsed after carcinogen treatment and mammary tumor promotion by pregnancy in Sprague-Dawley rats.

lanthrene. The dose used in these two sets of experiments was 10 mg 3-MCA daily for 6 or 10 days administered by intragastric intubation. Rats were then mated 4 days after the last dose of the carcinogen. The results clearly show that pregnancy after the administration of a chemical carcinogen induced a significant increase in tumor incidence, a greatly shortened latent period and a marked increase in the number of tumors developed. Thus, whereas 3-MCA given at 10 mg daily for 6 days induced a tumor incidence of only 10%, the same dose of the carcinogen caused a marked rise of tumor incidence to 50% with a latent period of 40 days, if the rats became pregnant after carcinogen treatment. Similar results were seen in rats given a higher dose (10 mg × 10) of 3-MCA. Again, one sees a significant increase in tumor incidence and greatly shortened latent period as a result of pregnancy. It is interesting to note that the latent period of tumor appearance was practically the same in all the pregnant groups, irrespective of the dose of carcinogen. Also, as the time between pregnancy and the last dose of the carcinogen increased, the latent period was further shortened (expt. V, Fig. 1). Parturition brought about a rapid regression of tumor growth, but subsequent pregnancy regularly led to the regrowth of these regressing tumors (6). Altogether, these experiments demonstrate the greatly increased hormonal activity during pregnancy that is a complex interaction of steroidal and polypeptide hormones and is the cause of the greatly accelerated tumor growth, since these tumors all regress after parturition.

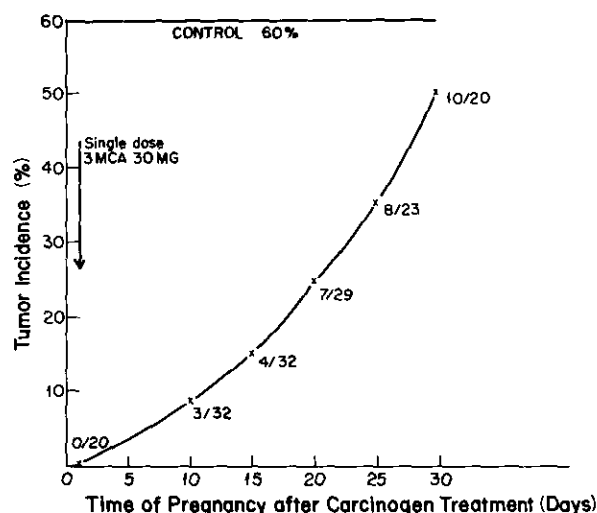The effect of pregnancy on tumor promotion, however, was abolished when the carcinogen was given to rats that were already pregnant. Thus, if 3-MCA, irrespective of dose (10 mg × 6, or 10 mg × 10), was given to female pregnant rats, mammary tumor induction was partially or totally inhibited (6,7). This failure to enhance tumor induction in rats fed 3-MCA during pregnancy suggests at least that an initiation process for induction of "latent tumor cells" by the carcinogen is necessary. This conclusion can be further substantiated by experiments using a single feeding of 3-MCA to female rats; at different time intervals these carcinogen-treated rats were mated and pregnancy ascertained. The results disclosed in Figure 2 conclusively show that pregnancy immediately following carcinogen administration appears to inhibit carcinogenesis. However, as the time interval between carcinogen treatment and pregnancy increased, mammary tumor incidence rose. This experiment clearly demonstrates the critical significance of the time between initiation and promotion in carcinogenesis. It is interesting to note that in these pregnant groups, if tumors developed, they appeared before parturition. It should be pointed out also that the failure of the carcinogen to induce mammary tumors in pregnant rats may also be due to an as yet unelucidated factor that pregnancy induces cellular changes in the mammary gland, rendering it resistent to the effect of a carcinogen.

Effect of Ovarian Hormones on Mammary Tumorigenesis in Ovariectomized Rats

The other experiment that demonstrates the promoting effect of hormones is the induction of mam-

mary tumors by a chemical carcinogen in ovariecto-mized rats. It is suggested that if ovarian hormones are essential for initiation of malignant transformation of mammary cells, a chemical carcinogen given to a castrated female rat will be unable to induce the development of mammary tumors, even if ovarian hormones are given later on. In contrast, one would expect mammary tumor development in these castrated and carcinogen-treated rats after treatment with ovarian hormones, if the hormonal effect is promotional. The experiments were therefore designed to investigate whether ovarian hormones are of critical importance in tumor initiation or whether they are merely tumor promoters. The experiment involved seven groups of rats. After oral feeding of a 10-mg dose of 7,12- dimethylbenz(a)-anthracene (7,12-DMBA) to all animals, group 1 was kept as a control throughout the experiment, and groups 2-7, each containing twenty 55-to-60-day-old female Sprague-Dawley rats, were castrated at intervals of 1, 3, 7, 15, 20 and 30 days, respectively. At 40-50 days after DMBA feeding, 10 rats from each group received a pair of ovarian grafts, and the other half of the animals served as controls. The rats in all these experiments were examined weekly for tumors. The experiments were terminated at the end of 6-8 months. At autopsy, tumors and ovarian grafts were excised and were fixed for histological sections.

The results summarized in Figure 3 show that the removal of ovaries immediately after the administration of 10 mg DMBA reduced the incidence of mammary cancer. The data clearly demonstrate that the incidence of mammary tumors depends on the time of interaction between ovarian hormones and the carcinogen-treated gland. The longer the duration, the greater is the neoplastic transformational and tumor development. Thus, castration 20-30 days after DMBA treatment had no effect on neoplastic transformation and tumor incidence, since later transplantation of a pair of functioning ovaries caused the development of the mammary tumors, with an incidence comparable to that of the controls.

Studies reported earlier by Dao (3,8) using male rats and with a similar experimental design disclosed that when castrated male rats bearing functional ovarian grafts were treated with a single dose of 3-MC, mammary tumors were induced in 65% of the rats so treated. If, however, the carcinogen was given to the castrated rats first and ovaries were transplanted 30 days later, the tumor incidence was only 10%. Altogether, these experiments give convincing evidence that the presence of ovarian hormones was a prerequisite for neoplastic transformation of the mammary cells exposed to a
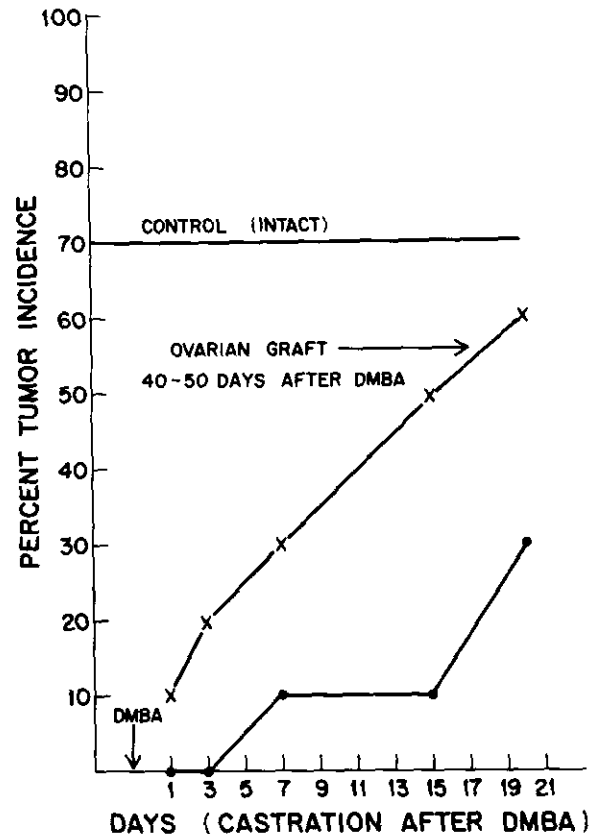

FIGURE 3. Mammary tumorigenesis in ovariectomized rats. See text for experimental procedure.

chemical carcinogen, since no mammary tumors appeared later even when the source of the ovarian hormones was restored.

Tumor Promotion in Mammary Carcinogenesis by Dietary Fat

The effect of high dietary fat on the development of spontaneous mammary tumors was reported more than three decades ago by Tannenbaum (9). Recent epidemiologic studies have led to a burst of investigation to examine the relationship between dietary fat and mammary tumors in experimental models. Thus, several investigators have reported that spontaneous, chemically or radiation-induced mammary tumors appeared early and in greater numbers in rats fed a high-fat diet compared to those fed a low-fat diet (10-12). Carroll and Khor further reported that enhancement of mammary tumor development was observed only in rats fed a high fat diet after but not before DMBA treatment (13). These authors concluded that a high-fat diet exerts its effect only during the promotional phase of mammary carcinogenesis. Subsequent investiga-

tions by others (14) largely using a similar experimental design appear to agree with the conclusion that high dietary fat promotes mammary tumor growth. Unfortunately, these conclusions were derived from experiments that were inadequately designed to study the role of dietary fat in mammary carcinogenesis. Careful examination of all these reported experiments, however, reveals at least one common deficiency in their experimental design. In all these studies, particularly those involving feeding a high-fat diet before carcinogen administration, the duration of feeding of the high-fat diet is often three to four times longer in rats receiving the high-fat diet after the carcinogen treatment than in those given the same diet before carcinogen administration. This enormous difference in the dietary fat intake is probably the major reason for the "erroneous" conclusion that the high-fat diet had no effect if given prior to carcinogen treatment. Re-

cent investigations from our laboratory disclosed results that strongly suggest that the tumor incidence in rats receiving the high-fat diet is a function of the duration of the high-fat diet feeding (5). Figure 4 demonstrates that mammary tumor incidence is positively correlated with the total time period of high-fat ingestion.

The efficacy of dietary fat in the enhancement of mammary tumorigenesis appears to be related to the amount of essential fatty acids in the dietary fat, as reported by Carroll and Khor (15). Saturated fats such as tallow and coconut oil do not contain sufficient essential fatty acids to support normal growth. Mammary carcinogenesis is reduced in rats fed diets containing these fats. But when 3% sunflower seed oil is added to a high-fat diet containing 17% coconut oil and fed to experimental rats, the diet enhances mammary carcinogenesis just as effectively as a high-fat diet containing 20% sunflower seed oil. These authors concluded that once the requirements for essential fatty acids are met, the total amount of fat intake, not the type of fat, is crucial in mammary tumor promotion (16). In contrast, we compared mammary carcinogenesis by NMU in four groups of Fischer rats fed a high-fat diet containing 32% corn oil, lard, coconut oil, or beef tallow supplemented with 1% corn oil. Mammary tumor incidence was significantly higher in rats fed the corn oil and lard diets than in rats fed the coconut and beef tallow diets (Table 1). Rogers and Wetsel (17) reported that a diet containing 30% beef tallow + 2% vegetable oil retarded mammary carcinogenesis by N-2-fluorenylacetamide (AAF) or DMBA, compared to a diet containing 15% vegetable oil. King et al. (18) demonstrated that mammary tumor incidence was lower in rats fed a diet containing 18% stripped hydrogenated coconut oil + 2% linoleic acid compared to rats on a 20% stripped corn oil diet. In the latter three experiments, there were sufficient amounts of essential fatty acids in the diets, yet rats on these diets exhibited reduced mammary tumor yield. Further studies are required to define whether the total amount of dietary fat or the type of fat is more important in mammary carcinogenesis.
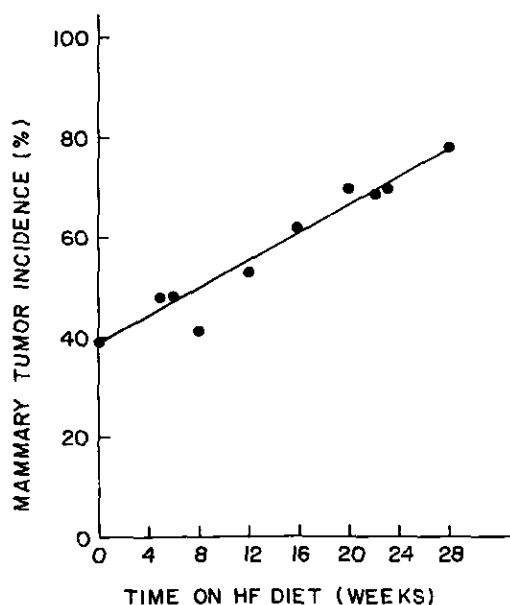

FIGURE 4. Relationship between mammary tumor incidence and duration of a high-fat diet feeding in female Fischer rats given N-methylnitrosourea at 50 days of age. Each group of rats was fed a high-fat diet for the duration of time indicated; otherwise, they were fed a low-fat diet.

Table 1. Mammary tumor incidence in Fischer rats fed different types of dietary fat.

Group	Diet	No. of rats at risk	Mammary tumor incidence, %	No. of tumors/rat
1	Corn oil	26	85%	1.5
2	Lard	27	63%	1.0
3	Beef tallow	30	50%	0.8
4	Coconut oil	23	43%	0.6

Relationship between Dietary Fat and Endocrine Functions

The possible modulation of endocrine functions by high dietary fat has been proposed by several investigators and data on the effect of a high fat diet on the levels of several hormones, including prolactin and estrogen, have been reported (11, 19-21). What is not clearly understood is the rationale or the conceptual basis for the suggestion that high dietary fat may modulate endocrine activities as the possible mechanism by which it enhances mammary tumorigenesis. However, since the effect of a high-fat diet is considered to be "promotional" and since hormones are known to be the key "promoting agents" of mammary tumor growth (22), it appears reasonable to suggest that the mechanism by which the high-fat diet enhances mammary carcinogenesis may indeed be the modulation of endocrine functions.

Effect of a High-Fat Diet on Serum Prolactin

Chan et al. (23) reported earlier that afternoon serum prolactin levels were significantly higher in proestrus rats on a high-fat diet than in rats on a low-fat diet. A paper published later by Ip et al. (21) reported that in rats with median eminence lesion, high-fat diets caused a significantly increased incidence of mammary tumors compared to those on a low-fat diet. The results suggested that prolactin was not a factor in enhancing mammary tumors in rats receiving a high-fat diet, since median eminence lesion induces elevated serum prolactin which is constant and not affected by other endocrine manipulations. Yet in the same paper, in sham-operated rats, a high-fat diet not only caused increased tumor incidence but also higher serum levels of prolactin than in those receiving the low-fat diet. These conflicting results were not convincingly explained.

The other indirect evidence suggesting a relationship between a high-fat diet and prolactin is the observation that enhancement of mammary tumorigenesis in rats fed a high-fat diet can be blocked by the administration of an ergot drug (CB-154) (24). It is known that ergot compounds inhibit prolactin secretion and reduce serum prolactin levels by probably both a direct effect on the pituitary and by increasing hypothalamic prolactin inhibiting factor.

The relationship between dietary fat and prolactin in mammary carcinogenesis is dubious at best. Recently, Hopkins et al. reported a lack of difference in serum prolactin levels in rats fed a high-

fat or low-fat diet (25). In all the above experiments, often only a single blood sample during the entire estrous cycle was assayed. These authors ignored the episodic fluctuations of prolactin levels and also the changes during the estrous cycles. Thus, data obtained from assay of a single blood sample at the end of the experimental period can hardly be valid for any meaningful interpretations. A carefully designed experiment is imperative to elucidate the possible influence of dietary fat on pituitary prolactin.

Effect of a High-Fat Diet on Serum Estrogens

Since prolactin synthesis and secretion by the pituitary is partially regulated by estrogen, the effect of the dietary fat on estrogen synthesis and secretion was investigated by Chan et al. (11). These authors reported that tumor-bearing rats on a high-fat diet have a slightly higher serum estrogen level than rats on a low-fat diet. Later, in our laboratory Ip observed that serum estrogen levels appeared to reflect the amount of fat in the diet (20). The data in both these experiments were again based on one single blood sample taken at the end of the experiment. Blood samples taken at regular intervals throughout a complete estrous cycle at varying time periods during the experiment may give a better picture of the effects of dietary fat on serum estrogen levels.

Indirect evidence suggesting a possible relationship between a high-fat diet and ovarian function was reported by Frisch et al. (26), who observed early vaginal opening and estrus in rats on a high-fat diet. This observation was confirmed by others (18) and led to the conclusion that a high-fat diet has an effect on ovarian function.

Discussion and Conclusion

Although hormones, particularly the ovarian hormones estrogen and progesterone, and the pituitary prolactin clearly play a major role in the promotion of mammary tumorigenesis induced by a chemical carcinogen, experimental results are equally convincing that the estrogenic hormone is critically needed for the initiation of neoplastic transformation of mammary cells. Our data disclose that mammary tumors do not develop if estrogen is absent during the initiation of neoplastic transformation by a carcinogen. Estrogen may act in a specific way to enhance the response of the interaction between the carcinogen and the target tissue in the initiation of carcinogenesis. Ovarian hormones play a dual function in mammary carcinogenesis.

The effect of dietary fat in mammary carcinogenesis, whether it is primarily tumor-promoting, remains to be investigated. The conclusion that high dietary fat only plays a role of a tumor promoter must be challenged, since the data are neither convincing nor properly interpreted. Earlier studies by Carroll and Khor (2) disclosed that a high-fat diet fed to rats 4 weeks after carcinogen treatment was unable to "promote" tumor growth. These authors reported that a high-fat diet was effective in enhancing mammary tumorigenesis only if it was given within 2 weeks of carcinogen treatment. These data are perplexing, in view of the data from studies of both mammary gland and skin carcinogenesis, which indicate that the tumor- promoting effect of hormones and croton oil (phorbol esters) could be demonstrated long after the administration of the carcinogen (27,28). If a high-fat diet is indeed a promoter, one would expect mammary tumor development, even if the high-fat diet was fed to rats long after carcinogen treatment.

Recent studies in our laboratory conclusively show that the mammary tumor yield in rats fed a high-fat diet was directly proportional to the time period of high-fat feeding. The longer the rats were fed a high-fat diet, the higher the mammary tumor incidence (15). This explains why enhancement of mammary carcinogenesis is more pronounced during the "promotional" phase as the rats were fed a high-fat diet continuously for a prolonged period of time until the end of the experiments. When the rats were fed a high-fat diet before carcinogen treatment, the maximal feeding duration was a 4-week period, since the experimental protocol calls for administration of the carcinogen at 50 days of age. In our experiments, when we fed rats a high-fat diet for 4 weeks after the carcinogen treatment, we failed to observe an increase in mammary tumor incidence in these rats compared to those rats fed a low-fat diet. Further experiments are required to clarify the mode of action of a high-fat diet in the enhancement of mammary carcinogenesis.

Although indirect evidence has been observed to suggest a relationship between a high-fat diet and endocrine functions, the data reported so far are unconvincing and confusing.

It must be noted that the role of prolactin, estrogen and progesterone in mammary carcinogenesis is interrelated; measurement of serum levels of these hormones will not elucidate the mechanism by which a high-fat diet affects mammary carcinogenesis. The biosynthesis of estrogen and progesterone in rats fed a high-fat diet must be investigated. Equally important is the study to determine whether the effects of a high-fat diet in mammary carcinogenesis are mediated via neuroendocrine mechanisms.

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